present Amendment as incorporating a Petition for Extension of Time for the appropriate length of time. Accordingly, any deficiency in extension fees required under 37 C.F.R. §1.17 may be charged to Deposit Account No. 13-2725.

In the Office Action mailed July 12, 1999 in the present case, the Examiner made the election/restriction requirement final; rejected claims 17-33, 39 and 41-42 as indefinite under § 112; rejected claims 17-33 and 41 as being unpatentable under § 103(a) over *Widder et al.* and *Connelly et al.*; and rejected claims 17-33, 38, and 41-42 as being unpatentable under § 103(a) over *Widder et al.* in view of *Kemmner et al.* and *Terasaki et al.* Applicants respectfully disagree with each of these determinations and request reconsideration.

The Examiner rejected claims 17-33, 39 and 41-42 under 35 U.S.C. § 112, second paragraph, as being indefinite. Applicants have amended claim 17 to more distinctly claim the subject matter of the invention. Support for the amendment can be found throughout the specification, and no new matter has been added. Applicants have amended the claim solely to address the Examiner's § 112 concerns, not to overcome any art of record, and the amendment should be viewed accordingly. Additionally, support for new claim 43 is found throughout the specification, and no new matter has been added.

The Examiner rejected claims 17-33 and 41 under 35 U.S.C. § 103(a) as being unpatentable over *Widder et al.* (EP 016,552) and *Connelly et al.* Applicants respectfully disagree and request reconsideration. Each of these references lacks elements of the present invention, and combining the references does not cure these deficiencies. Therefore, even if one of skill in the art were to combine these references, he or she would not achieve the present invention.

In order to establish a prima facie case of obviousness, three basic criteria must be met, namely: 1) there must be some suggestion or motivation to modify the reference or to combine the references; 2) there must be a reasonable expectation of success; and 3) the prior art reference (or references when combined) must teach or suggest all of the claim limitations. MPEP § 2142. Applicant submits that *prima facie* obviousness has not been established, as the cited references do not teach or suggest all of the claim limitations. The presently claimed invention provides a method for detecting a specific target cell in a cell suspension of a mixed cell population, in a fluid system containing a mixed cell population, or in a single cell suspension prepared from

solid tissue. None of the references, alone or in combination, teaches or suggests the present invention.

The Examiner conceded that the method of *Widder* differs from the instant invention in failing to teach incubation of the antibody coated microspheres in mild detergent for 5 to 10 minutes to 2 hours at 4° C. Further, the Examiner stated that *Widder et al.* fail to teach the use of an antibody to immobilize antibodies on the surface of the magnetic particles. However, in addition to these deficiencies, *Widder et al.* fail to teach detection of individual target cells as claimed in the present application. *Widder et al.* discloses a method for coarse separation of blood cells, and not detection of individual target cells.

Widder discloses microspheres having protein A associated with the outer surfaces thereof. These microspheres are not uniform regarding size and amount of protein A on the surface. Therefore, when the antibodies bind to protein A, one cannot know the amount of antibody per particle and the exact orientation of the antibody (see pages 4 and 7 of the reference). Moreover, due to the use of protein A, the microspheres of *Widder* will adhere to non-target cells and target cells alike and cause unacceptable reduction in specificity. In addition to binding the Fc portion of IgG cells, protein A will also bind to B cells and plasma cells present in a sample. As a result, *Widder* cannot achieve the present invention, since this method cannot be as specific as the presently claimed method.

Additionally, the method taught by *Widder* cannot achieve the sensitivity required by the present invention. The content of iron in *Widder's* microspheres varies. The magnetic strength of the microspheres is not powerful enough to pull a few target cells out of a population of several million cells. This results in a loss of sensitivity such that a fraction of the target cells will not be isolated, which is unacceptable for the presently claimed method.

In contrast, the present invention teaches a method for detecting a specific target cell in a cell suspension of a mixed cell population, in a fluid system containing a mixed cell population, or in a single cell suspension prepared from solid tissue. The method involves coating paramagnetic particles or beads with an antibody or antibody fragment directed against a membrane structure specifically expressed on the target cell and not on non-target cells in the cell mixture. As a result, any type of non-specific binding, such as that taught in *Widder* is unacceptable in the present invention.

Moreover, even if a person with knowledge in the art should consider using monclonal antibodies on the particles, on which *Widder* used protein A, he or she would still expect that the method would have the same disadvantages typical for *Widder's* method. The Examiner stated that it would have been obvious to one of ordinary skill in the art to immobilize other antibodies on the surface of the magnetic particles in the method of *Widder*. However, one of skill in the art would not expect to achieve the present invention by modifying *Widder*. The present inventors have surprisingly succeeded in avoiding the unspecific binding characterizing *Widder* by using the presently claimed method. The inventors further use the particle-target-cell complex to visually detect the cells using a microscope. This type of detection has not been accomplished previously, since it has not been possible to obtain particle binding exclusively to target cells. In fact, the present inventors encountered criticism from colleagues at the Norwegian Radium Hospital that the present invention would suffer the same disadvantages as encountered in *Widder et al.* Thus, it would not have been obvious to one or ordinary skill in the art to modify *Widder* to achieve the present invention.

side-by-side comparison? needed

Widder et al. separates cells when only two types of cells are present by using radioactive chromium, while the idea of the present invention is to detect individual target cells, the detection being done by visualizing the target cell rosettes under a microscope. The present inventors have surprisingly succeeded in avoiding the unspecific binding characterizing the other cited methods. Further, the inventors can use the particle-target-cell complex to visually detect two cells under the microscope. This was not possible previously, because it had not been possible to obtain particle binding exclusively to a specific target-cell.

The Examiner cited *Connelly* has teaching various fixatives used to fix cells without destroying cellular properties. However, *Connelly* has several deficiencies, and the combination of *Connelly* with *Widder* does not cure these deficiencies.

Connelly teaches a cell fixative composition for fixing the internal components of a cell without disrupting the cell surface components. *Connelly* uses fixatives without destroying the cells structurally, but these cells were killed. The present invention examines live cells, and these cells can be viewed in a microscope without using fixatives at all. The present method uses live cells in the selection and detection steps. The present method suggests optionally using fixatives if in special cases further visualization or characterization steps after detecting the cells

claims not limited to live cells

are warranted. Again, *Connelly* fails to teach or suggest a method of detecting specific target cells in a cell suspension.

The Examiner further stated that it would have been obvious to one of ordinary skill in the art to use detergents to treat cells used by *Connelly* following certain specific temperature and time parameters because the use of detergents to treat cells is well known and conventional in the art for removing extraneous matter from the cells that will interfere with assays. However, the use of detergents does not necessarily increase the specificity of the method. Therefore, persons with knowledge in the art would not consider using detergents since it would not make *Widder's* method more specific. In fact, the inventors of the presently claimed invention attempted to use detergents without obtaining sufficient specificity. However, use of a combination of detergents and low concentration and low temperatures surprisingly gave the specificity of the presently claimed method.

Therefore, even if one of skill in the art were to combine the teachings of *Widder* and *Connelly*, he or she would not achieve the present invention. The references do not teach or suggest, alone or in combination, a method for detecting a specific target cell in a cell suspension of a mixed cell population, in a fluid system containing a mixed cell population, or in a single cell suspension prepared from a solid tissue.

The Examiner rejected claims 17-33, 38 and 41-42 under 35 U.S.C. § 103(a) as being unpatentable over *Widder et al.* in view of *Kemmner et al.* and *Terasaki et al.* *Widder* is discussed above and Applicants refer to the above discussion as it applies to this rejection. Again, *Kemmner* and *Terasaki* fail to cure the deficiencies of *Widder*. Therefore, even if one of skill in the art were motivated to combine these references, he or she would not achieve the present invention.

The Examiner cited *Kemmner* as teaching isolation of tumor cells from a mixed cell suspension of human tumor tissue which contains tumor cells, leukocytes, and erythrocytes, using magnetic beads coated with monoclonal antibodies. The Examiner cited *Terasaki* as teaching the preparation of monoclonal antibodies for use in the diagnosis of neoplastic conditions, with a wide variety of different tumors.

Kemmner teaches the use of beads for enriching a cell population prepared from a solid tumor containing mainly tumor cells, and the reference uses the bead/cell complex to assess the

effects of the enrichment. However, only 96% of the bead-rosetting cells with the specific antibody proved to be tumor cells and 5% of the cells attached to the beads coated only with an irrelevant antibody bound to tumor cells. Moreover, of the 34% cells that bound control beads coated with an antibody recognizing the human leukocyte common antigen (Dako-LC), as much as 35% turned out to be tumor cells. This is a good example of the unspecificity of *Kemmner's* method since leukocyte common antigen is not expressed on the tumor cells. These data demonstrate a highly unspecific method which cannot be used to specifically and reliably detect target cells in a mixed cell population. Therefore, *Kemmner* also fails to teach or suggest a method for detecting a specific target cell in a cell suspension.

Terasaki teaches using hybridoma cell lines and does not refer to binding antibodies to cells. Further, *Terasaki* teaches detection of free antigen in the blood. In this case, all cells are removed from the sample. Therefore, *Terasaki* adds nothing to the other references in this case.

The present invention provides a method for detecting a specific target cell in a population of millions of cells. None of the references, alone or in combination, teaches or suggests the method as claimed. *Widder* simply teaches a coarse separation of blood cells which is nonspecific and provides low sensitivity. Moreover, *Kemmner* also suffers from great non-specificity. Finally, the addition of *Terasaki* with the above references does nothing to cure the deficiencies.

In view of the amendments and remarks presented herein, Applicants respectfully submit that the claims are in condition for allowance. Notification to that effect is earnestly solicited. If prosecution of this case could be facilitated by a telephonic interview, the Examiner is encouraged to call the undersigned.

Respectfully submitted,

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